

In re Patent Application of:

Daniell

Serial No.: **10/520,204**

Filed: **08/26/2005**

In the Claims

Please cancel the indicated claims without prejudice and substitute the pending claims as set forth below in a complete listing. Language added is shown underlined and language deleted is shown in strike through or enclosed in brackets. The amendments include no new matter and are fully supported in the application as filed.

1.(currently amended) A ~~plastid~~ transformation vector effective for stably transforming a plastid genome, said vector comprising, as operably-linked components, a first flanking sequence, at least one DNA sequence coding for a mercuric ion reductase (merA) and an organomercurial lyase (merB), a sequence encoding an antibiotic-free selectable marker, and a second flanking sequence, ~~wherein a plant is stably transformed with said plastid transformation vector, and said plant is capable of phytoremediating a contaminant compound.~~

2.(currently amended) The vector of claim 1, wherein said at least one DNA sequence is a ~~phytoremediation~~ phytoremediation operon.

3.(original) The vector of claim 1 or 2 further comprising a regulatory sequence.

4.(original) The vector of claim 3, wherein said regulatory sequence comprises a promoter operative in said plastid genome.

5.(original) The vector of claim 4, wherein said promoter is 16srRNA.

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6.(original) The vector of claim 3, wherein said regulatory sequence comprises a 3' untranslated region (UTR).

7.(currently amended) The vector of claim 1, wherein the vector is competent for ~~stabling~~ stably integrating in the plastid genome of a plant species and wherein the flanking DNA sequences are substantially homologous to sequences in a spacer region of said plastid genome, ~~and wherein said flanking sequences are conserved in the plastid genome of said plant species.~~

8.(original) The vector of claim 7, wherein said spacer region is a transcriptionally active spacer region.

9.(currently amended) The vector of claim 1, wherein the plastid ~~genome~~ is selected from ~~the group consisting of~~ a chloroplast, a chromoplast, an amyloplast, a proplastide, a leucoplast and an etioplast.

10.(cancelled)

11.(original) The vector of claim 1. wherein said first flanking sequence is trnI, and wherein said second flanking sequence is trnA.

12.(original) The vector of claim 11, wherein trnI and trnA provide for homologous recombination to insert an operon coding for a protein suitable for inactivating a contaminant compound into the spacer region in an inverted repeat region of a chloroplast genome.

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13.(currently amended) The vector of claim 1, wherein said ~~operon~~ vector is located in a single copy region of said plastid genome.

14.(currently amended) The vector of claim 6, wherein said 3'UTR 3' UTR is a 3'UTR 3' UTR of psbA.

15-16.(cancelled)

17.(currently amended) The vector of claim ~~46~~ 1, wherein said antibiotic-free selectable marker is ~~Betaine~~ betaine aldehyde dehydrogenase (BADH).

18-19.(cancelled)

20.(currently amended) A method for producing at least one DNA sequence coding for a protein ~~suitable~~ effective for inactivating a contaminant compound, the method comprising:

integrating the plastid transformation vector of claim 1 into the plastid genome of a plant cell; and

growing said plant cell to thereby express said ~~at least one heterologous~~ DNA sequence ~~coding for a protein suitable for inactivating a contaminant compound~~.

21.(original) The method of claim 20, wherein said at least one DNA sequence coding for a protein suitable for inactivating a contaminant compound is competent to phytoremediate a contaminant compound.

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22.(original) A plant stably transformed with the transformation vector of claim 1.

23.(currently amended) A progeny of the plant of claim 22, said progeny being stably transformed with said vector.

24.(currently amended) A seed of the plant of claim 22, the seed containing said vector.

25 (currently amended) A plant part of the plant of claim 22, ~~comprising a plastid including said at least one heterologous DNA sequence coding for a protein suitable for inactivating a contaminant compound~~ the plant part containing said vector.

26.(previously presented) A plant that comprises at least one chloroplast transformed with the vector of claim 1.

27.(previously presented) The plant of claim 26. wherein said plant further comprises a plurality of said chloroplasts in mature leaves.

28.(previously presented) The plant of claim 26, wherein said plant further comprises a plurality of said chloroplasts in young leaves.

29.(currently amended) A plastid transformation vector effective for stably transforming a plastid genome, comprising, as operably-linked components, a first flanking sequence capable ~~for~~ of integrating said plastid transformation vector into the plastid genome, an operon comprising *merA* and *merB* genes, a sequence encoding a

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marker selected from an antibiotic-free marker and an antibiotic resistance marker, and
a second flanking sequence capable for of integrating said plastid transformation vector
into the plastid genome.

30.(original) The plastid transformation vector of claim 29, wherein said first and
second flanking sequences allow site-specific integration of the operon containing the
merA and *merB* genes into an inverted repeat region of the plastid genome between *tml*
(tRNA Ile) and *trnA* (tRNA Ala) genes.

31.(currently amended) The plastid transformation vector of any one of claims 29 or
30, wherein said operon further comprises ~~the~~ an *aadA* gene as the selectable marker.

32.(currently amended) The plastid transformation vector of claim 29, further
comprising a 3' untranslated region (~~3'UTR~~) (3' UTR) positioned downstream of the
operon, and upstream of said second flanking sequence.

33.(currently amended) The plastid transformation vector of claim 32, wherein said
~~3'UTR~~ 3' UTR is from a *psbA* chloroplast gene.

34.(currently amended) A method of detoxifying mercury, said method comprising
~~the steps of:~~ integrating the vector of claim ~~45~~ 1 into a plastid genome of a plant cell,
culturing said plant cell to express *merA* and *merB*, and exposing said plant ~~cells~~ cell to
mercury.

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35.(currently amended) The vector of claim 2, wherein the operon is the *merAB* operon.

36.(currently amended) A plant cell ~~comprising~~ containing a plastid including an expression cassette, ~~said expression cassette comprising~~ having as operably linked components, a promoter functional in said plastid, ~~an operon encoding a merAB operon, a transcription termination region, a sequence encoding an antibiotic-free selectable marker,~~ and DNA sequences flanking the expression cassette ~~to facilitate stable integration of~~ and effective for stably integrating said expression cassette into a genome of said plastid ~~by homologous recombination.~~

37-40.(cancelled)